STUDIES ON THE PHYSIOLOGICAL EFFECTS OF HYDROGEN CYANIDE.

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The question of the physiological effects of cyanide has been of considerable interest since Claude Bernard (1) in 1857 noticed that the venous blood of vertebrates is bright red after treatment with cyanide. It is well known that cyanides in dilute solutions act in general as protoplasmic depressants. In most cases this depressing effect can be attributed to the inhibition of oxidations. Investigations by Allen (2), Child (3), Hyman (4, 4*a*, 4*b*), Vernon (5), Buchanan (6) and others show that potassium cyanide, even in extremely dilute solutions, depresses reversibly the rate of respiration in *Planaria*. Lund (7), however, noted no decrease in oxygen consumption in *Paramecia* placed in potassium cyanide solutions. The fact that dilute solutions of cyanide act as anesthetics is equally well known (Heilbrunn, 8; Osterhout, 9). An important difference between the effects of a typical anesthetic, such as ether, and cyanide was pointed out by Heilbrunn (8) who showed that ether decreases the viscosity of protoplasm while KCN in anesthetic concentrations increases it. Heilbrunn, therefore, concluded that in the case of sea urchin eggs there are two types of anesthesia; in one the viscosity of the cytoplasm is decreased and in the other it is increased. The toxic action of cyanide in concentrated solutions is also well established (Hyman, 4). In vertebrates the toxicity of cyanide seems to be due to its effect on the central nervous system, as shown by Geppert (10) and Dontas (11). Child (12) showed that the portion of an organism with the highest rate of metabolism is most susceptible to cyanide, and that young organisms having a high rate of metabolism are more susceptible than adults with lower rates. For a detailed review of the literature on the various phases of the cyanide problem, the reader is referred to the paper of Hyman (4).

The question of the effect of cyanide on the permeability of membranes is a debatable one. It is generally considered that anesthetics, such as alcohol and ether, decrease permeability (Lillie, 13; Lullies, 14; McClenden, 15; Osterhout 9*a*). Wertheimer (16), on the other hand, concluded that narcotics increase the permeability of frog skin while Krehan (17) showed that KCN increases the permeability of plant cells to many substances.

Most workers with cyanide have used potassium cyanide, which in an aqueous solution is strongly alkaline, due to the manner in which it dissociates:

$$\begin{array}{c} KCN + H_2O \rightleftharpoons KOH + HCN \\ \Updownarrow \\ K + OH \end{array}$$

Hydrogen cyanide in an aqueous solution acts as an extremely weak acid, dissociating only to a slight degree. In view of the fact that the question of the effect of cyanide on permeability is a debatable one and since most of the previous workers have used KCN, it was thought advisable to study in detail the penetration of hydrogen cyanide through living membranes as well as its effect on the membrane.

The investigations were conducted at the Zoölogical Laboratory, University of Pennsylvania, for which privilege the writer wishes to acknowledge his indebtedness to Doctor C. E. McClung. The writer is also under obligations to Doctor J. H. Bodine, under whose direction the investigations were conducted, for many helpful suggestions throughout the progress of the work.

It was found convenient in this work to use the artificial "cell" devised by Jacobs (18) and constructed in the following manner: a hard glass tube 7 cm. long and 1.5 cm. in diameter was tapered at one end to an opening of one cm. in diameter and the tapered end provided with a tip. The skin from the hind legs or back of a freshly killed frog (*Rana catesbiana* or *R. pipiens*) was carefully stretched over the lipped end of the tube and held in place by a rubber band. The skin was so placed over the tube that the inside, or flesh side, of the skin was exposed to the exterior. The "cell" so constructed was placed in a 100 cc. quinine bottle and

both the " cell " and bottle fitted with rubber stoppers. The inside of the " cell " was filled with a borax-boric acid buffer solution and a solution of HCN in a borax buffer was placed in the quinine bottle. It was necessary to use a buffer which would not injure the skin or react chemically with either the cyanide or the silver nitrate solution used to determine the concentration of cyanide. The pH values from 6.8 to 9.2 were obtained by changing

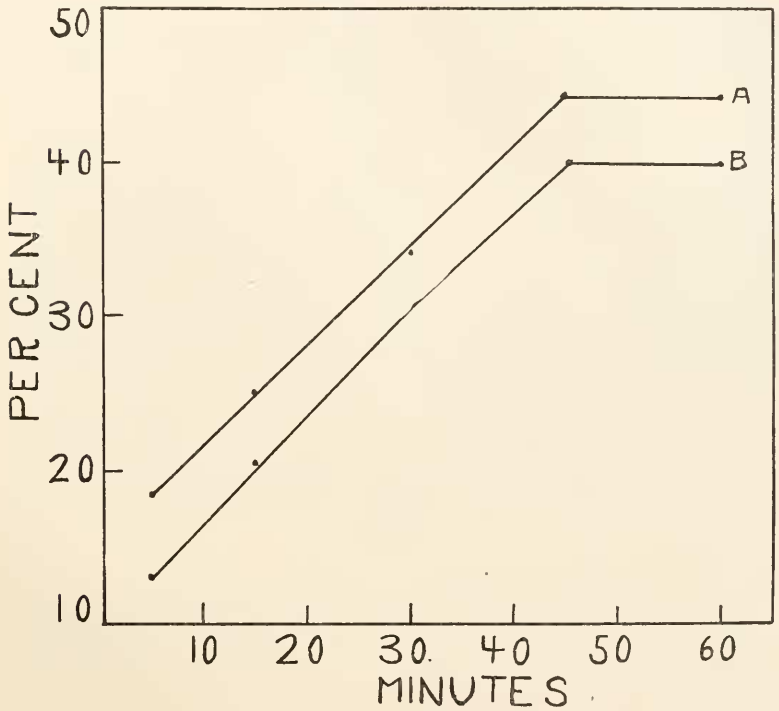

FIG. 1. Curve showing the relation of the position of the skin to the permeability of the skin to cyanide. *A*, skin with the flesh side out; *B*, skin normal, flesh side in. Abscissæ, time in minutes; ordinates per cent. cyanide.

the relative amounts of sodium borate and boric acid; for pH values below 6.8 it was necessary to add small amounts of nitric acid. The addition of HCN did not change the pH of the solution of either the borax or the borax plus nitric acid. Pure liquid hydrogen cyanide was used. No leakage occurred around the skin as shown by the fact that when a " cell " was filled with an

indicator and placed in a dilute solution of HCl the indicator did not change color. Several tests were conducted by reversing the skin and it was noted that the membrane was slightly more permeable to HCN when the skin was turned inside out than when it was in a normal position. The difference, however, was so slight that it is not significant (Fig. 1).

The "cells" after being filled with a borax buffer and put in a solution of HCN, were placed in a water bath at a constant temperature of 25° C. for one hour. At the end of that time equilibrium was reached between the cyanide inside and outside the cell. Five cubic centimeters of the internal and external solution were titrated with $N/50$ silver nitrate, using a one cc. pipette as a burette. The concentration of the cyanide solution used was $M/313$. That the skin was not killed at this concentration was easily demonstrated by substituting for the cyanide solution mineral acids, known not to penetrate living membranes. No change in intracellular acidity was noted in such control experiments. Experiments were conducted using external and internal solutions of various pH values, the external pH varied from 5. to 8.6 and the internal pH from 6.5 to 8.0. The results plotted in Fig. 2, show the relation of concentration of the total cyanide (HCN and CN) found in the cell at equilibrium to the various external and internal pH values. From this figure it may be noted that the penetration curve closely approximates the dissociation curve (x) and that the total concentration of cyanide inside the cell corresponds very closely to the undissociated cyanide in the external solution. The degree of dissociation represented in the curve was calculated from the formula

$$\log 1/H = \text{pH} = \log 1/K + \log a/1-a \quad (19),$$

where a = degree of ionization: K = dissociation constant, for HCN = 4.7×10^{-7} (20).

Figure 2 shows that at equilibrium, when the pH is from 5. to 5.5, the concentration of total cyanide within the cell is equal to the amount of cyanide in the external solution. From the dissociation curve it is evident that at this pH there is practically no dissociation, all of the cyanide being in the molecular condition. As the external pH was increased, the penetration curve rapidly

dropped. Likewise as the pH was increased the degree of ionization was increased. At a pH of 7.0 the dissociation is practically 50 per cent. and the concentration of cyanide in the cell is about 40 per cent. of the concentration of the external solution.

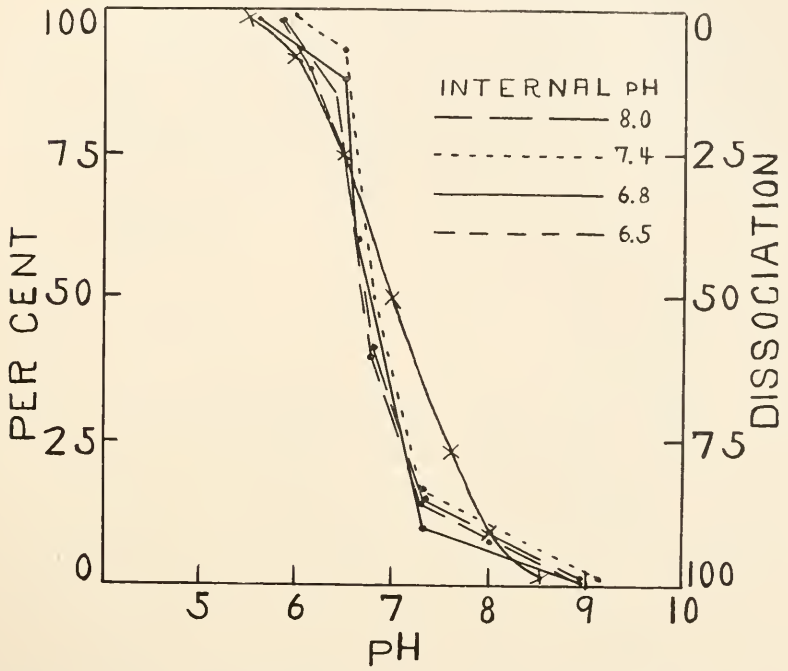

Fig. 2. Curve showing the effect of pH on the permeability of frog skin to HCN. Abscissæ represents the external pH; ordinates the per cent. of total cyanide on the right and the degree of ionization on the left. Calculated degree of dissociations represented by *X*. Internal pH values indicated as follows: short dashes 6.5, solid line 6.8, dotted line 7.4, long dashes 8.0.

At a pH of 9.0 dissociation is practically complete and accordingly little or no cyanide is present in the cell. It is evident that hydrogen cyanide seems to penetrate frog skin chiefly in the form of molecules and not as ions. It is also apparent from Fig. 2 that the intracellular pH varying from 6.5 to 8.0 does not affect the permeability of frog skin to hydrogen cyanide. Brooks (21) stated that changing the pH of the sap of Valonia with CO_2 and NH_3, changed the amount of 2 — 6 — dibromo phenol indophenol

that entered the cells. However, she states and also Scarth (22) shows that the pH of the sap in the vacuole could be no criterion of the pH of the protoplasm.

Experiments with Tadpoles, Daphnia and Elodea.

Experiments conducted with young bull frog tadpoles (Fig. 3) and *Daphnia* (Fig. 4) gave the same relative results as the experiments conducted with cyanide on frog skin "cells." After being separated from their cultural medium by cheese cloth, the organisms were placed in Syracuse watch glasses filled with a solution

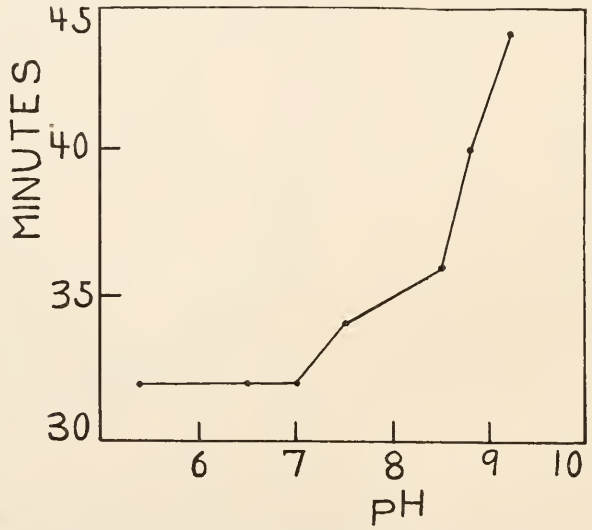

Fig. 3. Curve showing the effect of pH on the toxicity of HCN to young bullfrog tadpoles. Abscissæ represents the pH; ordinates the time in minutes required to produce death. Ten animals were used in each test.

of HCN in a borax buffer solution. It was previously determined that the animals were not killed in the buffer solution free of cyanide until after an exposure of three to four hours. On account of the anesthetic action of the cyanide, tadpoles did not prove to be good material for this work, since it was difficult to determine the time of death. When *Daphnia* was used the beat of the heart could be observed under a binocular microscope and the death point determined at the instant the heart stopped beating. It was noted, that the heart continued beating for some time after

other body movements had ceased and that the organism did not recover after the heart stopped.

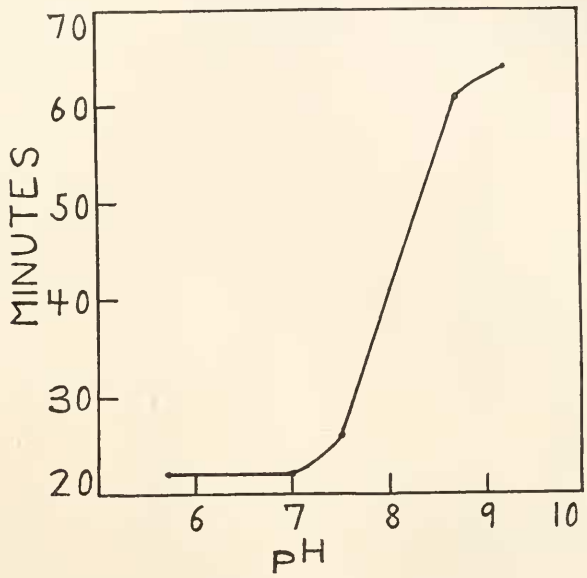

FIG. 4. Curve showing the effect of pH on the toxicity of HCN to *Daphnia*. Abscissæ represents pH; ordinates time in minutes required to kill 95 per cent. of the organisms.

It is obvious from Fig. 3 and 4 that less time was necessary to kill the animals in acid solution than in the alkaline solution. It required twenty-two minutes to kill *Daphnia* at a pH of 5.7 to 7.0; forty minutes at a pH of 8.0 and sixty-four minutes at a pH of 9.0. The concentration of cyanide used in the above experiment was $M/450$. The results indicated what would be expected from the study of the frog skin "cell," where more cyanide entered when the external pH was acid than when alkaline. The animals were killed first in solutions of the same pH values in which HCN penetrated the frog skin cells most quickly.

The effect of the pH on penetration of cyanide was further checked by studying its effect on the streaming of protoplasm in *Elodea* cells (in press). The streaming of the protoplasm can be observed under the high power of a microscope while the cells are immersed in a solution of cyanide. The pH was controlled,

as before, by a borax buffer. Leaves of *Elodea* near the growing tip of the branch were placed in a solution of hydrogen cyanide in Syracuse watch glasses and the time noted when the streaming of the protoplasm ceased. The results of a typical set of experi-

FIG. 5. Curve showing the effect of pH on the streaming of protoplasm in *Elodea* cells. Abscissæ represents pH; ordinates time in minutes required for streaming to stop.

ments are plotted in Fig. 5. Again the results show that the streaming of protoplasm stopped first in solutions of the same pH values in which HCN penetrated the artificial frog skin "cell" most quickly. Thus, in determining the toxicity of hydrogen cyanide to any organism, the value of controlling the hydrogen ion concentration of the solution is apparent.

It is obvious from the foregoing discussion that hydrogen cyanide penetrates living membranes chiefly in the form of molecules and in this respect is similar to other weak acids. Jacobs (18*a*) showed that carbonic acid killed various species of protozoa in a different order than mineral acids, which act primarily through their H ion (Collett, 23). This indicated to Jacobs, that the physiological effect of CO_2 was due to the entrance of the molecule. Beerman (24) and Bodine (25) obtained similar results with H_2S and HCN respectively. More recently, Osterhout (9*b*) and Osterhout and Dorcas (9*c*) showed by direct analysis that hydrogen sulfide and carbonic acid penetrated the living cells of *Valonia* chiefly in the form of molecules and not as ions. Brooks (21*a*) found that the amount of 2 — 6 — dibromo phenol indophenol in the sap of *Valonia* was proportional to the amount of undissociated dye in the external solution.

Effect of Temperature.

A series of experiments was conducted to determine the effect of temperature on the permeability of frog skin to hydrogen cyanide. The results are plotted in Fig. 6. It is evident from these curves that the higher the temperature the greater is the concentration of intracellular cyanide at any stated time. The curve suggests that of a typical unimolecular reaction as can be easily demonstrated by calculating the velocity constant from the following equation:

$K = 1/t \log a/a-x$ (26) in which $x =$ the amount of cyanide in the cell at any time t; $a =$ the amount of cyanide in the cell at equilibrium (Table I.).

Table I.

Velocity Constant K Calculated from the Unimolecular Equation $K = \frac{1}{t} \log \frac{a}{a-x}$ at Various Temperatures.

External and internal pH 6.8.

6°.				16°				25°.			0°.				34.5°.		
t	a	x	k	t	a	x	k	t	a	x	k	t	a	x	k	t	a	x	k
Min.																			
15	1.84	.99	.0222	15	2.37	1.18	.0207	15	3.56	2.24	.0276	15	3.63	1.84	.0204	15	3.69	2.04	.0251
30		.158	.0256	30		2.17	.0357	30		3.11	.0252	30		2.97	.0256	30		2.90	.0223

It will be noted from Table I. that the velocity constant for any temperature is fairly consistent with the exception of 16°, and that K for the various temperatures is nearly constant. Plotting the log of the rate against the reciprocal of the absolute tempera-

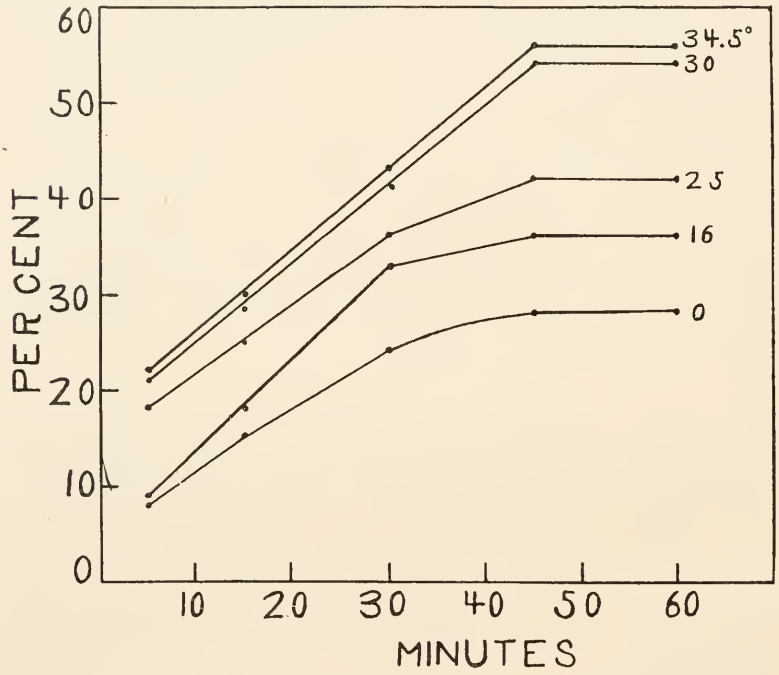

FIG. 6. Curve showing effect of temperature on the permeability of frog skin to HCN. Internal and external pH 6.8. Temperatures used were 0°, 16°, 25°, 30°, 34.5° C. Abscissæ represents exposure in minutes; ordinates per cent. of total cyanide.

ture, a curve was obtained as indicated in Fig. 7. The rate represents the time when the intracellular concentration of cyanide is twenty-five per cent. of the external concentration. It will be seen from this figure that there is a break in the line at 16° C. (.003415). Calculating Q from the Vant Hoff-Arhenius equation, (26)

$$K_2/K_1 = Q/2\left(\frac{T_2 - T_1}{T \cdot T_2}\right),$$

a value of 11,179 is obtained at a temperature from 16° C. to 34.5 and 4,300 for 0° to 16°.

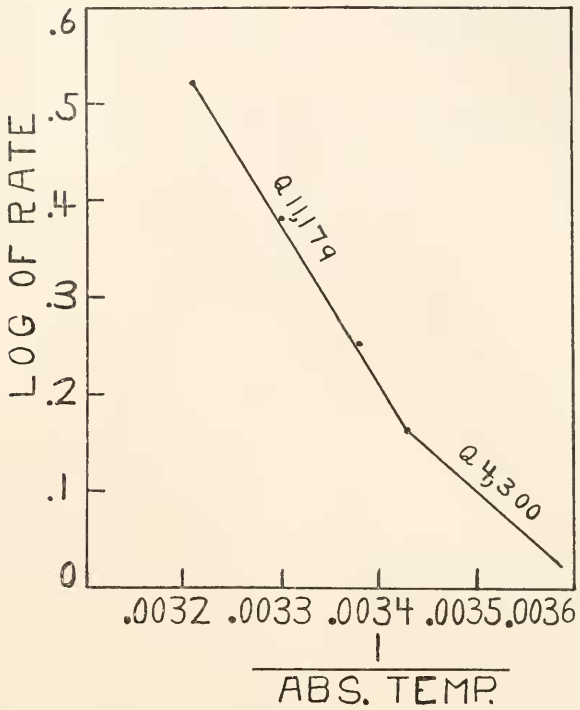

FIG. 7. Curve showing the log of the rate plotted against the reciprocal of the absolute temperature. Rate calculated from Fig. 5 as the time taken for the intracellular cyanide to equal 25 per cent. of the external concentration. Calculated from the formula

$$\frac{K_2}{K_1} = \frac{Q}{2}\left(\frac{t_2 - t_1}{t_2 \cdot t_1}\right).$$

Abscissæ represents reciprocal of the absolute temperature. Ordinates, the log of the rate.

RELATION OF CONCENTRATION.

Experiments were undertaken to determine the effect of the concentration of cyanide in the external solution on the penetration of hydrogen cyanide through frog skin. The external and internal pH and the temperature were maintained constant at 6.8 and 25° C. respectively. The concentrations of cyanide were *M*/109, *M*/124, *M*/160, *M*/196, *M*/225 and *M*/313. The results of such an experiment are plotted in Fig. 8. Each point on the

curve represents the average of three to five tests. It may be seen from this figure that, with the exception of the two low concentrations ($M/225$ and $M/313$), the total amount of cyanide within the cell at equilibrium was the same for all the concentrations. The rate of entrance of cyanide increased with an increase in

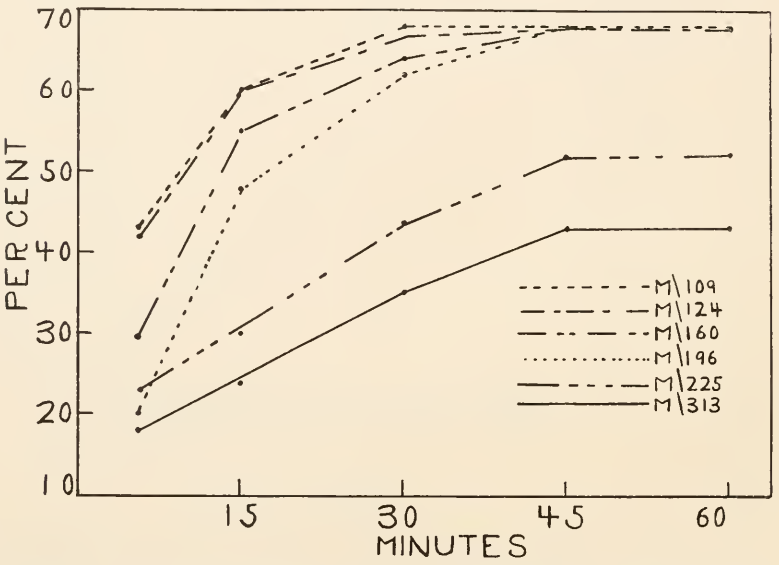

FIG. 8. Curve showing the effect of concentration of HCN upon the permeability of frog skin to cyanide. Concentrations of HCN used were $M/109$, $M/124$, $M/160$, $M/196$, $M/225$, and $M/313$. Abscissæ represents the time in minutes; ordinates per cent. cyanide.

concentration. At a concentration of $M/109$, equilibrium was reached within 30 minutes, while at lower concentrations equilibrium was not reached for a period of 45 minutes. The fact that the skin is not killed can be proved by substituting for the cyanide solution a mineral acid, which is known not to pass through living membranes, and testing the pH of the internal solution. As there is no change in the intracellular acidity, it is evident that no acid has passed through the skin.

Although, it is known that the frog skin is not killed by the above treatment with HCN, it is desirable to ascertain what effect the cyanide does have upon it. A series of experiments was conducted to determine the effect of hydrogen cyanide on the po-

tential difference of frog skin and the results are given below in detail.

Effect of Hydrogen Cyanide on the Potential Difference of Frog Skin.

Osterhout (*9c*) and others have shown that the electrical resistance of an organism is an excellent indicator of its vitality and that death is accompanied by an increase in permeability. An increase in permeability is equivalent to an increase in the electrical conductivity or to a decrease in resistance. In order to determine the physiological effect of hydrogen cyanide on frog skin, it was

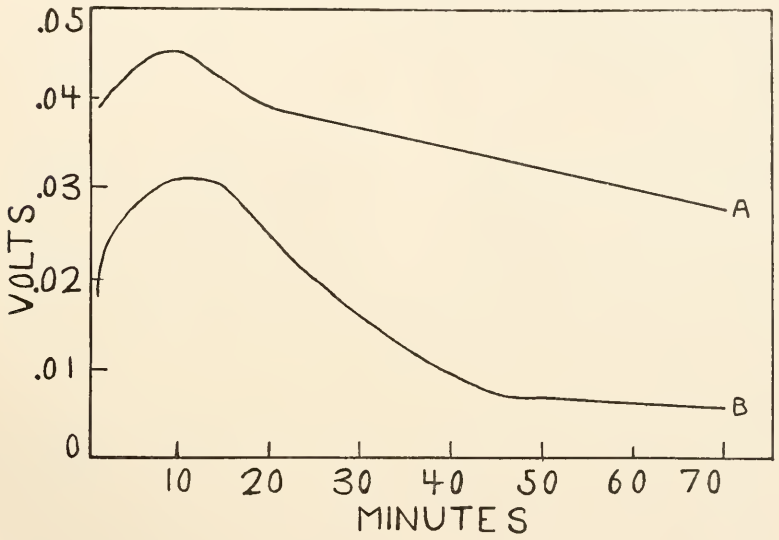

Fig. 9. Control experiments; curve showing the P.D. of frog skin in *A*, Ringer's solution and *B*, borax-boric acid buffer. Abscissæ represents the exposure in minutes. Ordinates P.D. in volts. Different pieces of skin from the same frog were used.

potentiometer. The same solution was placed inside and outside the cell. The skin from the hind legs of bull frogs (*Rana cates-* thought advisable to study its potential difference before and after being immersed in various solutions of this chemical. The apparatus was similar to that previously used in the first part of this paper with the exception that non-polarizable zinc electrodes were placed inside and outside the cell and the potential read on a

biana) was used. Since the potential difference of the skin from different frogs and also different pieces of skin from the same frog, varied considerably, it was necessary to repeat each experiment many times and only characteristic curves of single experiments will be given. There is some evidence at hand to indicate that the conditions under which the frogs are kept influences to some extent the potential difference of the skin. It was observed in several instances, when the temperature of the vivarium dropped several degrees below normal, that the potential difference of the skin also dropped and that on warm days the potential difference was usually higher than that at other times.

Control experiments were conducted by placing the skin in solutions of borax-boric acid buffer and Ringer's solution. The result of a typical control test is given in Fig. 9. Readings on the potentiometer were taken every minute. The temperature varied during the tests from 20–21° C. The experiments were run in parallel series using different pieces of skin from the same animal. It will be noted from Fig. 9, that there was an initial rise in P.D. followed by a gradual decline. The two curves are approximately parallel indicating that the borax buffer is no more toxic to frog skin than is the Ringer's solution. The pH of the Ringer's solution was about 8.2 and the borax 6.8.

A series of experiments was undertaken to determine the relation of the concentration of cyanide to the potential difference of frog skin. The cyanide solution was made by adding pure liquid HCN to a borax buffer at a pH of 6.8. The concentrations of cyanide used were $M/136$, $M/154$ and $M/225$. Fig. 10 shows the results of a typical set of experiments. The cells were placed in a borax buffer for ten minutes, then removed and placed in the cyanide solution. The skin was allowed to remain in the cyanide solution for various periods of time, then removed and placed in a borax buffer free from HCN. The period between arrows indicates the time that the cells were exposed to cyanide. In all cases it may be observed that the skin completely recovered after being removed from the cyanide solution. After the cells had been removed from the borax solution and placed in cyanide solution at a concentration of $M/225$, a great stimulation occurred, followed by a gradual drop in the potential difference to the base line. This

stimulation, characterized by a rise in potential difference, is also evident at a concentration of $M/154$ but not nearly to the same degree as with the weaker concentration of cyanide. The drop following stimulation at a concentration of $M/154$ is practically of the same magnitude as obtained with a $M/225$ solution. In the case of the $M/136$ solution there was no stimulation but the potential difference dropped suddenly to the base line. The skin

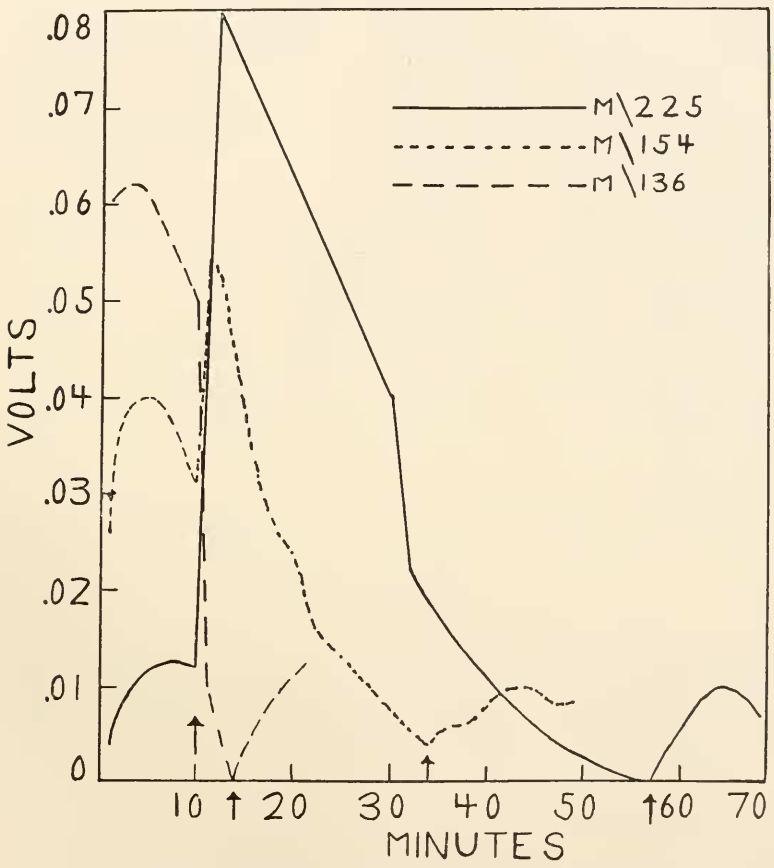

FIG. 10. Curve showing the relation of concentration of HCN to the P.D. of frog skin. Concentration of total cyanide = $M/225$, $M/154$ and $M/136$. Same solution on both sides of the membrane. The period between arrows indicates the time that the skin was exposed to cyanide, at other times the skin was exposed to borax buffer free from HCN. Abscissæ represents the time in minutes; ordinates the P.D. in volts. Different pieces of skin from the same frog were used.

recovered when removed from the cyanide. It is obvious from Fig. 10, that a weak solution of cyanide acted at first as a stimulant and this was followed by a delayed toxicity. As the concen-

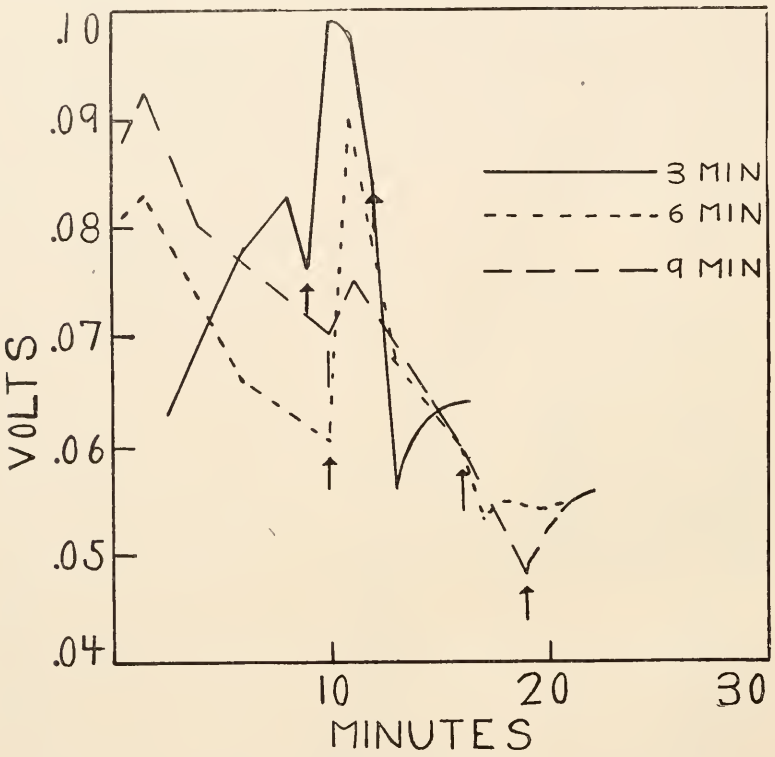

FIG. 11. Curve showing the effect of short exposures of cyanide to the P.D. of frog skin. The time between arrows represents the period of exposure to HCN, at other times the skin was exposed to borax buffer alone. Solid line represents a three minute exposure to cyanide; dotted line, 6. min. exposure and broken line 9 min. exposure. Abscissæ represents the exposure in minutes; ordinates, the P.D. in volts.

tration of cyanide was increased, the stimulation decreased and the toxic effect increased until a point was reached where stimulation no longer occurred and at this point the toxic action was pronounced. This initial stimulation of frog skin by cyanide was apparently overlooked by Lund (*7a*) who took readings every fifteen minutes. He did notice, however, a rapid rise followed by a rapid fall in the electrical resistance in Obelia during a period

of exposure to KCN. It is well known that many anesthetics in dilute solutions act as protoplasmic stimulants and in concentrated solutions their toxic action is established (Osterhout, 9).

It is interesting in this connection to determine whether the rapid fall in the potential difference immediately following stimulation by dilute cyanide is due to a natural recovery to normal or to the toxic action of the cyanide. Cells were removed from the borax buffer solution at the end of a ten minute exposure and placed in a hydrogen cyanide solution of a concentration of $M/160$. A series of three experiments was conducted; in the first series the cells were removed from the cyanide solution at the end of three minutes and placed in a borax buffer, at which time the stimulation had reached its maximum and started to drop. In the second series the skin was allowed to remain in the cyanide solution for six minutes, then removed and placed in the pure buffer, at which time the drop following stimulation had reached the same reading as when the skin was first placed in the cyanide. In the third series the skin was exposed to the cyanide for a period of nine minutes. At the end of that time, the drop following stimulation had reached a point below the original P.D. The results of a typical series of experiments are plotted in Fig. 11. It is evident from this figure that after the removal of the skin from the cyanide solution, in the three and six minute exposure, the drop in P.D. continued until it had fallen below the P.D. obtained at the time the skin was placed in the cyanide solution; the P.D. then increased to normal. The nine minute exposure showed no further drop in the P.D. after being removed from the cyanide solution but an immediate recovery occurred. It is apparent from the data given that the drop in potential difference immediately following the stimulation was due not to the toxic action of the cyanide but to a natural return to the original reading and the toxic action did not take place until after the drop had surpassed the point where the skin was stimulated.

Effect of the pH of Cyanide Solution on the Potential Difference.

It is apparent from the data given in the first part of this paper that little or no HCN penetrates living membranes except in the

form of undissociated molecules; thus it was deemed advisable to study the effect of dissociated and undissociated molecules of HCN on the potential difference of frog skin. Control experiments were conducted using borax-boric buffer free from cyanide at a pH of 6.8 and 8.5 to determine whether or not the hydrogen ion has any effect on the frog skin. Fig. 12 shows the results

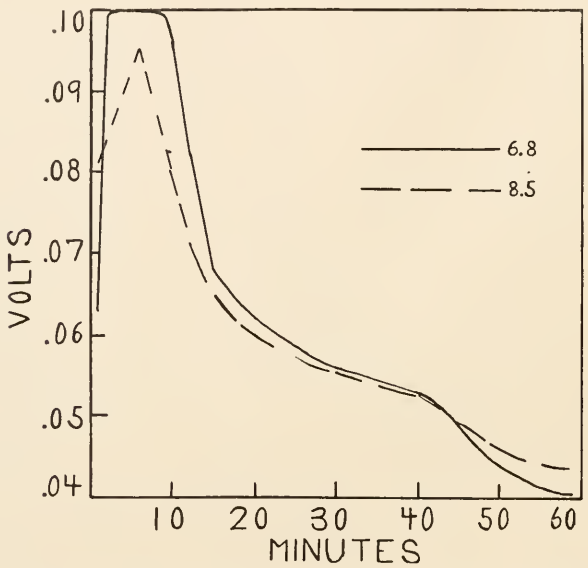

Fig. 12. Curve showing the effect of pH of the borax buffer on the potential difference of frog skin. pH valves 6.8 and 8.5. Abscissæ represents the exposure in minutes; ordinates, the P.D. in volts.

obtained. It is obvious that the two curves coincide very closely and that the hydrogen ion had no effect on the skin. It may be noted that the curves are strikingly similar to the control curves plotted in Fig. 9. The initial rise followed by a gradual decline was again manifested. Fig. 13 shows the results obtained by placing the frog skin "cells" in a solution of HCN in borax buffer at pH values of 6.8 and 8.5. The concentration of cyanide used was M/154. The temperature was constant at 22° C. ± 0.5°. The "cells" were placed for ten minutes in a borax buffer solution at pH values of 6.8 and 8.5 respectively; at the end of that time they were removed and put in a borax buffer containing

HCN at their respective pH values. The period of exposure to was greater at a pH of 8.5 than at 6.8. It is also apparent that the drop in the P.D. after a return to normal was somewhat more cyanide is represented on the curve by the time between the arrows. From Fig. 13, it is obvious that the initial stimulation pronounced under the acid conditions than under the alkaline.

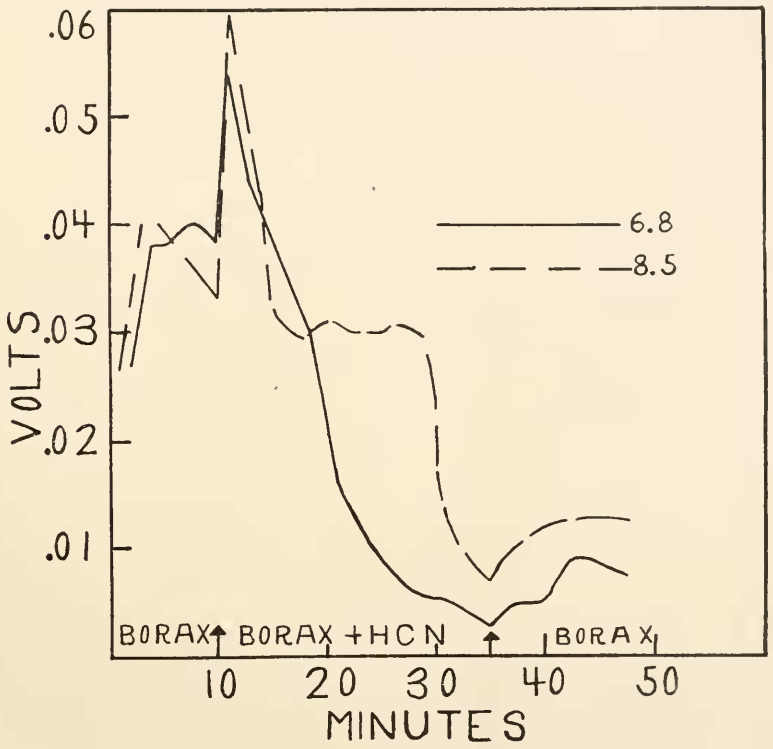

Fig. 13. Curve showing the effect of the pH of a solution of HCN in borax on the P.D. of frog skin. Period of exposure to cyanide represented as the time between arrows, at other times the skin was exposed to borax alone. pH values used were 6.8 and 8.5. Abscissæ represents the exposure in minutes and the ordinates the P.D. in volts.

There actually are fewer molecules of cyanide in a solution with a pH of 8.5 than with a pH of 6.8 (Fig. 1). So if the molecules were the toxic units, it would be expected that there would be less stimulation and more toxicity when the solution contains the greatest number of molecules than under the reverse conditions of

less molecules and more ions. Such a condition was found to exist, as is evident from Fig. 13 where more stimulation and less toxicity occurred under the alkaline conditions than in the acid solutions.

In Fig. 14 are plotted the results of a series of tests using a solution of HCN in Ringer's solution. The pH of Ringer's solution was about 8.2; by the addition of HCl the pH was changed to

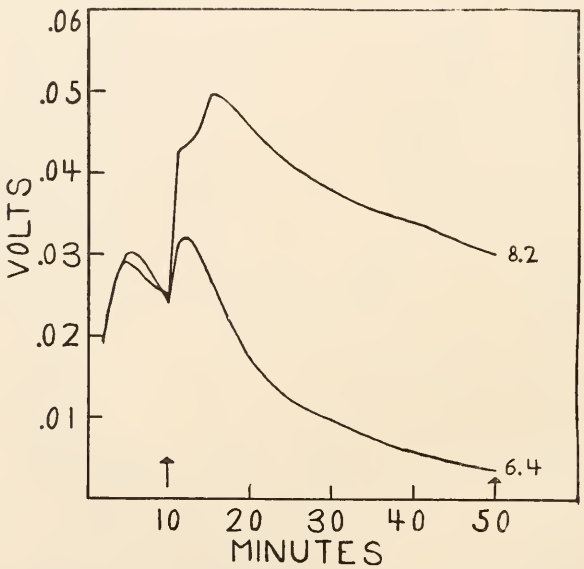

FIG. 14. Curve showing the effect of the pH of a solution of HCN in Ringer's solution. Exposure to cyanide represented by the time between arrows. First ten minutes skin exposed to Ringer's solution alone. pH values used were 6.4 and 8.2. Abscissæ represents the exposure in minutes and the ordinates the P.D. in volts.

6.4. The same concentration of cyanide was used in each series ($M/250$). The "cells" were placed in Ringer's solution for ten minutes, then removed; one series was placed in a solution of HCN in Ringer's solution at a pH of 6.4 and the other series at a pH of 8.2. Potentiometer readings were taken every minute for 45 minutes. Again it is evident from Fig. 14, that there is greater stimulation and less toxicity under alkaline than acid conditions. In either case the initial stimulation was not as pronounced as in the experiments when borax buffer solution was used. It is, how-

ever, sufficiently marked to be significant. Figs. 15 and 16 show the results obtained when the pH was alternated from 6.6 to 8.2 and vice versa. The same piece of skin was used under the same conditions of temperature and concentration of cyanide; the concentration of cyanide was $M/173$, made by adding liquid HCN to

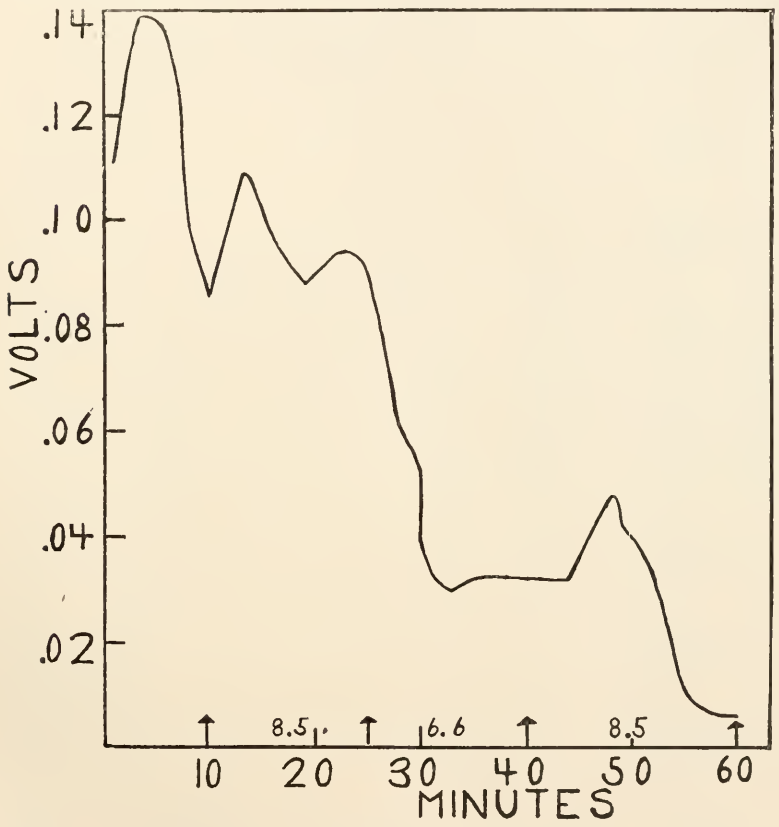

FIG. 15. Curve showing the effect of alternating the pH of a solution of HCN in borax on the same piece of frog skin. pH changed from 8.5 to 6.6 then back to 8.5. Period of exposure at various pH value indicated in curve. For the first ten minutes the skin was exposed in minutes and the ordinates, the P.D. in volts.

a borax buffer. The skin was placed in a borax solution for ten minutes, then removed and placed in the cyanide solution at a pH of 8.5 for 15 minutes (Fig. 15). The characteristic curve was obtained—an increase in P.D. followed by a gradual decrease.

When the period of 15 minutes had elapsed, the skin was removed and placed in a HCN solution of the same concentration and at a pH of 6.6. It will be noted from Fig. 15 that a very sudden drop occurred in the P.D. as soon as the skin was placed in the solution at a pH of 6.6. At the end of 15 minutes the skin was again placed in the original HCN solution at a pH of 8.2.

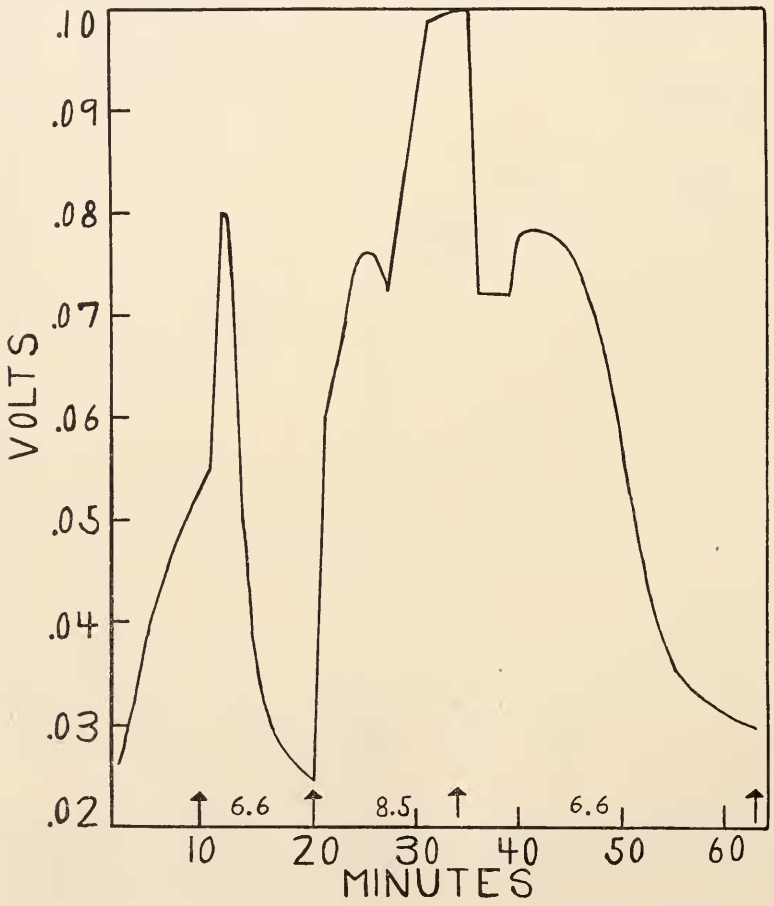

FIG. 16. Curve showing the effect of alternating the pH of a solution of HCN in borax on the same piece of frog skin. PH changed from 6.6 to 8.5 then back to 6.6. Period of exposure at various pH values indicated in curve. For the first ten minutes the skin was exposed to borax alone. Abscissæ represents time of exposure in minutes and the ordinates the P.D. in volts.

The P.D. gradually increased—suggesting a recovery—followed by a gradual decrease to the base line. The curve suggests, that at a pH of 6.6 the cyanide was more toxic than at a pH of 8.2 as was evident from the fact that the P.D. suddenly dropped when the skin was taken from a solution of a pH of 8.2 and placed in a pH of 6.6. When the skin was taken from a pH of 6.6 and placed in a solution of 8.5 a recovery occurred.

The conditions plotted in Fig. 16 are the reverse from those in Fig. 15. The skin after being placed in a borax buffer solution for ten minutes was removed and put in a cyanide solution of a pH of 6.6. The initial rise followed by a rapid drop was again obtained. When, however, the skin was removed from the pH of 6.6 and placed in a cyanide solution having a pH of 8.5, the P.D. rapidly increased until a point was reached above the initial stimulation suggesting a recovery and a stimulation. At the end of 15 minutes the skin was again placed in the original HCN solution at a pH of 6.6; a rapid fall occurred, suggesting a return to normal, followed by a rapid decline. As previously stated, 98 per cent. of the total cyanide is dissociated at a pH of 8.5 and thus the cyanide is present in the ionic condition. When the pH is 6.6 the cyanide is about 40 per cent. dissociated, therefore, the number of molecules and ions is about equal. From the data given, it appears that the cyanide is more toxic to the frog skin when the solution is acid than when alkaline. Thus, it seems that the molecule is actually more toxic than the ion. In dilute concentrations the physiological effect of hydrogen cyanide on frog skin is first a stimulation followed immediately by a rapid return to the original reading terminating in a toxic effect which will eventually prove fatal.

Summary.

The experiments indicate that little or no HCN penetrates frog skin "cells" except in the form of undissociated molecules. The total amount of intracellular cyanide is proportional to the concentration of undissociated molecules in the external solution. The internal pH value of the "cell" has no effect on the penetrations of HCN through frog skin.

From a study of the effect of hydrogen cyanide on the potential

difference of frog skin, it appears that dilute solutions of cyanide cause an initial stimulation followed by toxicity. As the concentration is increased, the stimulation is decreased and the toxicity is increased until a certain concentration of cyanide is reached where there is no stimulation but a marked toxic effect is evident.

The data also indicate that the molecule is more toxic than the ion.

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